Effects of pH and Acid Resistance on the Radiation Resistance of Enterohemorrhagic *Escherichia coli*

ABSTRACT

The effects of pH and the induction of pH-dependent stationary-phase acid resistance on the radiation resistance of *Escherichia coli* were determined for seven enterohemorrhagic strains and one nonenterohemorrhagic strain. The isolates were grown in acidogenic or nonacidogenic media to pH levels of approximately 4.7 and 7.2, respectively. The cells were then transferred to brain heart infusion (BHI) broth adjusted to pH 4.0, 4.5, 5.0, and 5.5 (with HCl) that was preequilibrated to 2°C, and cultures were then irradiated using a ^{137}Cs source. Surviving cells and the extent of injury were determined by plating on BHI and MacConkey agars both immediately after irradiation and after subsequent storage at 2°C for 7 days. Decreasing the pH of the BHI in which *E. coli* was irradiated had relatively little effect on the microorganism's radiation resistance. Substantial differences in radiation resistance were noted among strains, and induction of acid resistance consistently increased radiation resistance. Comparison of *E. coli* levels immediately after irradiation and after 7 days of refrigerated storage suggested that irradiation enhanced pH-mediated inactivation of the pathogen. These results demonstrate that prior growth under conditions that induce a pH-dependent stationary phase cross-protects *E. coli* against radiation inactivation and must be taken into account when determining the microorganism's irradiation *D* value.

The reduction of pH is the primary means of preventing the growth of human pathogens for a wide range of fermented and acidified ready-to-eat foods. However, recent disease outbreaks associated with highly infectious acid-tolerant microorganisms have shifted the microbiological concerns associated with these acidic products from preventing microbial growth to ensuring the absence or inactivation of these pathogens. This is particularly true for enterohemorrhagic *Escherichia coli* (EHEC). Low levels of EHEC surviving in moderately acid products such as fermented sausages, apple juice and cider, yogurt, and mayonnaise have been the cause of several outbreaks of hemorrhagic colitis *(4, 9, 10, 34, 36, 43, 46)* which included cases that resulted in hemolytic uremic syndrome and deaths. Recent studies have demonstrated that *E. coli* O157:H7 can survive for extended periods in moderately acidic products (pH 3.5 to 5.5), particularly when held at refrigeration temperatures *(11, 13, 14, 20, 23, 33, 39, 45, 46, 47)*. Further, the results of a recent survey of ready-to-eat fermented sausage products suggest that the pH and water activity of a substantial portion of these products are insufficient to ensure inactivation (and in some cases to prevent growth) of EHEC *(27)*. However, many of these products have sensory properties that are sensitive to heat, and alternatives to thermal processing are needed to ensure elimination of acid-tolerant pathogens.

Low-dose irradiation is an effective means of eliminating *E. coli* O157:H7 and other foodborne pathogens from raw meat and poultry, with the specific dose required being primarily dependent on the temperature of the product during irradiation (i.e., refrigerated versus frozen) *(2, 11, 12, 16, 19, 37, 41, 42)*. However, there is relatively little information available on its efficacy in moderately acidic products or on how radiation resistance of enteric foodborne pathogens is influenced by pH. Potentially, irradiation and a reduced pH could act synergistically to enhance the inactivation of the microorganism; however, past research with *E. coli* is inconclusive. Bànà et al. *(2)* concluded that the radiation resistance of *E. coli* was unaffected when the organism was irradiated in chicken meat modified to have pH values between 3.7 and 5.5. Conversely, Fielding et al. *(16)* reported that irradiation *D* values were similar to those for *E. coli* in chicken were obtained when *E. coli* was irradiated in yeast extract glucose broth at pH 7.0 to 4.3 kGy, but there was a significant reduction in radiation resistance when the medium was adjusted to pH 4.1 or 4.0.

Interpretation of past research on the sensitivity of foodborne pathogens to the combined effects of irradiation and acidic pH levels may be confounded by two factors. The first is the effect of temperature during and after irradiation. In addition to influencing radiation resistance *(41)*, temperature also has a strong influence on the sensitivity of foodborne pathogens to pH inactivation *(6, 7, 8, 23, 45, 47)*. Second, there has been no evaluation of the potential impact that acid resistance may have on radiation resistance. Foodborne pathogens such as *E. coli, Shigella* spp., *Salmonella* spp., and *Listeria monocytogenes* have inducible and phase-dependent mechanisms for surviving expo-

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TABLE 1. Escherichia coli *strains used in study*

Strain designation	Strain	Serotype	Source and acid resistance[a]
S1[b]	B1409[c]	O157:H7	Clinical isolate, Centers for Disease Control and Prevention (CDC). Acid tolerance Induced: extreme Uninduced: strong
S2	45753-35	O157:H7	Isolated from beef kidney sample, USDA Food Safety and Inspection Service (FSIS). Acid tolerance Induced: extreme Uninduced: strong
S3	30-2C4	O157:H7	Clinical isolate from dry cured salami outbreak, CDC via FSIS. Acid tolerance Induced: extreme Uninduced: extreme
S4	932	O157:H7	Clinical isolate, CDC via M. P. Doyle, University of Georgia. Acid tolerance Induced: extreme Uninduced: moderate
S5	Ent-C9490	O157:H7	Clinical isolate from "Jack-in-the-Box" outbreak, CDC. Acid tolerance Induced: extreme Uninduced: extreme
S6	A9124-C1	O157:H7	Clinical isolate, CDC. Acid tolerance Induced: extreme Uninduced: moderate
S7	95JB1	O111:H$^-$	Clinical isolate from mettwurst outbreak, Adelaide Children's Hospital (Australia) via J. Paton. Acid tolerance Induced: extreme Uninduced: extreme
S8	ATCC 25922	Not available	Reference strain, Difco. Acid tolerance Induced: extreme Uninduced: weak

[a] Based on survival at 37°C in BHI adjusted to pH 2.5 using HCl *(5)*. pH-dependent stationary-phase acid resistance was induced or not induced by growing the cultures in an acidogenic or nonacidogenic medium, respectively *(5)*. Extreme, <1-log reduction after 7 h; strong, 1- to 2-log reduction after 7 h; moderate, 2- to 4-log reduction after 7 h; weak, >3-log reduction after 2 h.
[b] Strain designation used for convenience in the current manuscript.
[c] Actual strain designation.

sure to both moderate and strongly acidic conditions *(1, 3, 5, 8, 18, 21, 25, 26, 29, 40)*. Induction of acid resistance can also have wide-ranging effects on the ability of bacteria to resist other stresses such as heating, antimicrobials, and exposure to UV light *(15, 22, 28, 30, 35)*. However, it appears that no studies have examined the ability of acid resistance to cross-protect foodborne pathogens from the effects of ionizing radiation.

The goal of the current study was to acquire basic, quantitative data that are needed to use irradiation as an alternative means of controlling EHEC in moderately acidic foods. Using a microbiological medium with pH adjusted by the addition of HCl as a model system, the specific objectives of the study were to (i) characterize the effect of pH (4.0 to 5.5) on radiation resistance, (ii) determine if irradiation enhances acid inactivation during postirradiation refrigerated storage, (iii) evaluate the effect of pH-dependent stationary-phase acid resistance on radiation resistance, and (iv) assess the effects and interactions of irradiation, moderately acidic pH, and acid resistance on the extent of injury.

MATERIALS AND METHODS

Microorganisms. The study was carried out using seven EHEC and one non-EHEC strains (Table 1) so the biological variability associated with observed responses could be evaluated. The acid resistance of these strains had been previously characterized *(5)*. All strains were transferred bimonthly by culturing in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) for 24 h at 37°C. These working stock cultures were stored at 2°C.

Cells induced and noninduced for pH-dependent stationary-phase acid resistance were obtained by growing the microorgan-

isms in tryptic soy broth with 1% (wt/vol) dextrose (TSB+G) and tryptic soy broth without dextrose (TSB−G), respectively, according to the technique of Buchanan and Edelson *(5)*. These cultures were incubated without agitation for 18 h at 37°C. The initial pH of both media was 7.0 to 7.2, whereas the final pH of the TSB+G and TSB−G cultures was 4.6 to 4.7 and 7.0 to 7.2, respectively.

Preparation of culture for assessment of radiation resistance. Brain heart infusion (BHI) broth with concentrated HCl added to attain pH values of 4.0, 4.5, 5.0, and 5.5 was selected as the model system in which the *E. coli* O157:H7 cells were irradiated. BHI broth was selected because it has been used extensively a model system for meats. HCl was used as the acidulant for modifying the pH of BHI broth in order to limit the effects observed to those caused by pH, thus avoiding confounding the interpretation due to the uptake of organic acids being dependent on the molecule being present in an undissociated form and the anion effects associated with various organic acids *(6, 7)*. The BHI broth was dispensed in 5.0-ml portions to test tubes (16 by 125 mm) and the tubes sealed with plastic caps and sterilized by autoclaving for 20 min at 121°C. The pH values of representative BHI tubes were determined after autoclaving to verify that the pH was unchanged. All BHI tubes were preequilibrated to a temperature of 2°C. This temperature was carefully maintained (± 0.5°C) throughout subsequent inoculation, irradiation, and sampling to eliminate temperature as a potential confounding factor.

For each irradiation dose to be tested, three BHI tubes for each pH level were placed around the circumference of a circular plastic test tube rack (total of 12 tubes per rack). A second test tube rack contained an identical set of 12 tubes. All BHI tubes were inoculated with 0.2 ml of the 18-h TSB+G or TSB−G culture of one of the eight *E. coli* strains. The initial population density was approximately 2×10^7 CFU/ml. One rack was designated as containing the time-0 samples, which were analyzed immediately after irradiation. The test tubes from the second rack were held for 7 days after irradiation at 2°C before being analyzed. The test tubes in each rack were inoculated just prior to being irradiated to minimize the time between inoculation, irradiation, and determination of survivors. Typically, the time between inoculation and plating of the irradiated samples was less than 1 h. Controls (0.0 kGy) were handled in an identical manner except that they were not irradiated.

Irradiation of cultures. The racks of BHI tubes were irradiated individually to the specified dose using the cesium-137 self-contained gamma radiation source at the USDA Eastern Regional Research Center. The dose rate was 0.102 kGy/min, which was established using National Physics Laboratory dosimeters and corrected monthly for decay of the source. Sample temperature was maintained in the irradiator by thermostatically controlled injection of the gas phase from liquid nitrogen into the circular sample chamber. Temperature was monitored continuously and maintained at 2°C (±0.5°C).

Determination of survivors. The time-0 samples were analyzed immediately after irradiation by diluting appropriately, using 9.9-ml dilution blanks of sterile 0.1% (wt/vol) peptone (Difco) water and then surface plating on duplicate prepoured plates of brain heart infusion agar (BHIA; Difco) and MacConkey Agar (MA; Difco) with use of a spiral plater (Model 3000, Spiral Biotech, Bethesda, Md.). All plates were incubated for 18 to 20 h at 37°C and then enumerated using an automatic plate counter (Spiral Biotech). The lower limit of detection was 10 CFU/ml.

The dual plating system was used to determine the extent of injury. The BHIA counts provided the total number of viable cells while the MA counts provided the number of noninjured cells. The ratio of noninjured to injured bacteria was calculated by the difference between BHIA and MA counts: log(injured cells) = log(BHIA count) − log(MA count).

The BHI tubes that were stored at 2°C for 7 days after irradiation were analyzed as above.

Calculation of irradiation *D* values. The radiation resistances of the strains were quantified by calculating irradiation *D* values (radiation dose that produces a 90% reduction in the number of survivors). An irradiation *D* value is the negative reciprocal of the slope of the survivor curve. The slopes were determined by linear regression. There were at least three replicates (i.e., three tubes) for each irradiation dose for each survivor curve.

RESULTS

Radiation resistance. While some survivor curves showed evidence of small shoulders or tails, the irradiation inactivation of *E. coli* was considered to follow first-order kinetics. Linear regressions of the log of the number of surviving cells versus radiation dose had R^2 values >0.90, with most survivor curves having R^2 values >0.96 (Table 2). Examples of typical survivor curves are depicted in Figure 1.

Radiation resistance varied among the strains; irradiation *D* values ranged from 0.05 to 0.18 kGy (Table 2). The most obvious factor influencing resistance was whether the strain had been grown in acidogenic TSB+G (final pH 4.6 to 4.7) or nonacidogenic TSB−G (final pH 7.0 to 7.2) prior to being irradiated in pH-modified BHI broth. The *D* values for *E. coli* strains grown in TSB+G were as much as two-fold greater than the *D* values for the corresponding TSB−G-grown cells. The magnitude of the increase in *D* values varied among the strains. With the exception of strains S4 and S5 the increase in resistance as a result of prior growth in acidogenic TSB+G was greater as the pH of the BHI broth in which strains were irradiated was decreased.

In addition to enhanced radiation resistance as a result of prior acid habituation of the strains, a number of other effects were noted (Table 2). When initially grown in TSB−G, strains with moderate (S4, S6, and S8) and strong (S1 and S2) acid resistance were less radiation resistant than the strains having extreme acid tolerance (S3, S5, and S7). This relationship between pH-independent acid resistance and radiation resistance was not evident when pH-dependent stationary-phase acid resistance was induced by prior growth in TSB+G, though S5 and S7 continued to have the greatest *D* values. The effect of BHI pH on the radiation resistance of EHEC varied with strain and prior growth conditions, and overall had a relatively minor effect on radiation resistance. For the TSB−G cultures, *D* values tended to decrease to a small degree for all strains as the pH became more acidic. This small increase in radiation sensitivity with decreasing pH of the suspending menstruum was observed with strains S1, S4, S5, S6, and S7 when they were cultured initially in TSB+G, but not strains S2, S3, and S8.

The current study also evaluated the possibility that, in

TABLE 2. *Effect of pH and prior growth in media that did and did not induce pH-dependent stationary-phase acid resistance on the irradiation D values of enterohemorrhagic* Escherichia coli *suspended in brain heart infusion broth acidified with hydrochloric acid*

		TSB+G-grown cells[b]		TSB−G-grown cells[b]		
Strain[a]	pH	*D* value[d] (kGy)	R^2 value	*D* value[d] (kGy)	R^2 value	Resistance ratio[c]
S1	4.0	0.12	0.97	0.07	>0.99	1.8
	4.5	0.12	0.97	0.07	>0.99	1.8
	5.0	0.12	0.98	0.08	>0.99	1.6
	5.5	0.17	0.99	0.08	>0.99	1.5
S2	4.0	0.11	0.96	0.06	0.98	1.8
	4.5	0.11	0.97	0.07	0.99	1.7
	5.0	0.10	0.96	0.07	0.99	1.4
	5.5	0.09	0.94	0.07	0.99	1.2
S3	4.0	0.11	0.97	0.09	0.99	1.3
	4.5	0.11	0.96	0.09	0.99	1.2
	5.0	0.11	0.97	0.10	0.99	1.1
	5.5	0.11	0.97	0.11	0.98	1.0
S4	4.0	0.11	0.98	0.06	0.99	1.8
	4.5	0.12	0.98	0.06	>0.99	1.9
	5.0	0.12	0.99	0.07	0.99	1.9
	5.5	0.14	0.99	0.07	0.99	1.9
S5	4.0	0.15	0.97	0.10	0.98	1.4
	4.5	0.17	0.98	0.11	0.98	1.6
	5.0	0.16	0.98	0.13	0.98	1.2
	5.5	0.18	0.98	0.12	0.91	1.4
S6	4.0	0.06	0.94	0.05	0.98	1.4
	4.5	0.07	0.94	0.05	0.99	1.4
	5.0	0.07	0.94	0.05	0.99	1.4
	5.5	0.07	0.94	0.07	0.99	1.1
S7	4.0	0.15	0.96	0.10	0.98	1.6
	4.5	0.15	0.97	0.10	0.98	1.5
	5.0	0.16	0.97	0.12	0.99	1.3
	5.5	0.16	0.97	0.12	0.98	1.3
S8	4.0	0.13	0.98	0.07	0.99	2.0
	4.5	0.13	0.99	0.07	0.99	1.8
	5.0	0.13	0.97	0.09	0.99	1.5
	5.5	0.12	0.98	0.09	>0.99	1.4

[a] See Table 1 for strain identities.
[b] Induced or uninduced cells were obtained by growing them in acidogenic TSB+G (final pH 4.6 to 4.7) or nonacidogenic TSB−G (final pH 7.0 to 7.2), respectively, for 18 h at 37°C.
[c] D_{TSB+G}/D_{TSB-G}.
[d] The standard errors of *D* values were consistently less than 0.01 kGy.

addition to direct inactivation of EHEC, irradiation might potentiate (i.e., increase) acid inactivation of surviving cells during subsequent refrigerated storage. This was done by storing a second set of irradiated and unirradiated samples for 7 days at 2°C before determining viable counts. This temperature was selected to maximize the survival of *E. coli* under these moderately acidic conditions. Examples of the survivor curves encountered after 0 and 7 days of storage are depicted in Figure 2. The effect of pH alone was assessed by calculating the differential in log counts of surviving bacteria (i.e., BHIA counts) between day 0 and day 7 for the unirradiated control (0.0 kGy) samples (Table 3). Quantification of the combined effects of pH and irradiation on the survival of EHEC during refrigerated storage was calculated by the difference in the day-0 and day-7 viable counts based on the calculated irradiation dose needed to reduce the level of surviving *E. coli* in the day-7 sample to the lower limit of detection (log CFU/ml = 1.00) (Fig. 3). These values were determined by linear regression. Potentiation of inactivation during storage was then assessed by comparing this difference with that for the unirradiated (0.0 kGy) controls. If the difference in plate count values for the irradiated cultures was substantially greater than the corresponding difference for the unirradiated cultures, the irradiation treatment enhanced inactivation of the pathogen during refrigerated storage.

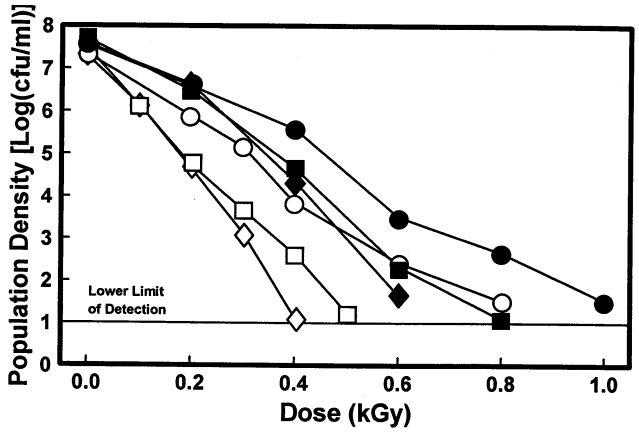

FIGURE 1. *Typical survivor curves of enterohemorrhagic* Escherichia coli *exposed to low-dose gamma irradiation at 2°C. Strain S1, pH 5.5: TSB+G-grown,* ■; *TSB−G-grown,* □. *Strain S2, pH 5.0: TSB+G-grown,* ●; *TSB−G-grown,* ○. *Strain S5, pH 5.0: TSB+G-grown,* ♦; *TSB−G-grown,* ◇.

The unirradiated, TSB+G-grown cultures decreased by

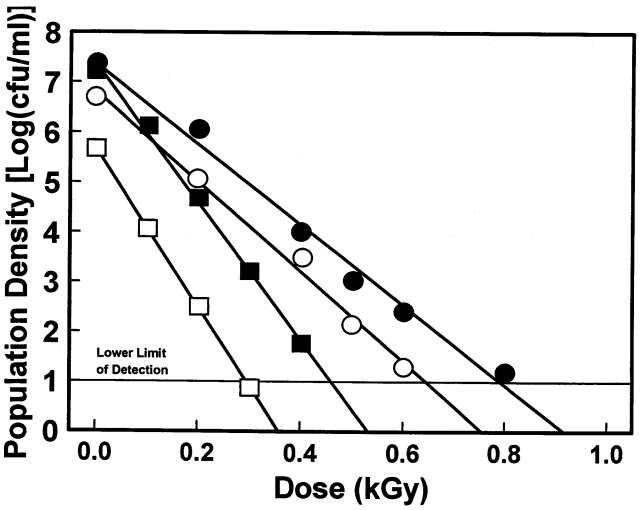

FIGURE 2. *Comparison of typical survivor curves observed immediately after irradiation treatment and after subsequent storage at 2°C for 7 days. Strain S2, TSB−G-grown, pH 5.5: time 0,* ■; *day 7,* □. *Strain S8, TSB+G-grown, pH 5.0: time 0,* ●; *day 7,* ○.

TABLE 3. *Effect of pH on the survival of irradiated and unirradiated enterohemorrhagic* Escherichia coli *in acidified brain heart infusion broth stored for 7 days at 2°C*

		Log change in BHIA count after 7 days			
		TSB+G-grown cells[b]		TSB−G-grown cells[b]	
Strain[a]	pH	Unirradiated[c]	Irradiated[d]	Unirradiated[c]	Irradiated[d]
S1	4.0	−0.03	+1.54	+0.64	+1.94
	4.5	+0.16	+0.26	+0.15	+0.97
	5.0	+0.29	+0.58	+0.34	+0.82
	5.5	+0.15	−0.25	+0.58	+0.57
S2	4.0	+0.19	+0.96	+0.18	+1.66
	4.5	+0.13	+1.09	+0.19	+1.05
	5.0	+0.34	+0.23	+0.75	+1.26
	5.5	+0.38	−0.49	+1.57	+2.30
S3	4.0	+0.08	+1.03	+0.10	+0.76
	4.5	+0.16	+1.02	+0.06	+0.19
	5.0	+0.10	+0.37	+1.01	+0.35
	5.5	+0.15	+0.65	+1.01	+0.01
S4	4.0	+0.04	+0.86	+0.34	+1.30
	4.5	−0.09	−0.08	+0.35	+0.67
	5.0	+0.20	+0.11	+0.93	+1.75
	5.5	+0.27	+1.09	+1.68	+1.96
S5	4.0	0.00	+0.80	+0.26	+0.22
	4.5	−0.04	+0.52	+0.29	+0.07
	5.0	−0.06	+0.36	+0.38	+0.36
	5.5	+0.01	+0.99	+0.61	+0.55
S6	4.0	−0.09	+0.95	+1.04	+0.67
	4.5	−0.08	−0.46	+0.08	+0.80
	5.0	−0.04	0.00	+0.39	+1.45
	5.5	+0.02	+0.67	+1.41	+2.42
S7	4.0	0.00	+1.46	+0.08	+1.20
	4.5	+0.15	+0.82	+0.11	+0.37
	5.0	+0.08	+0.80	+0.47	+1.10
	5.5	+0.11	+1.26	+0.64	+1.18
S8	4.0	+0.07	+1.39	+0.74	+0.94
	4.5	+0.08	+0.33	+0.50	−0.04
	5.0	+0.06	+0.18	+0.49	−0.17
	5.5	+0.05	0.00	+0.75	−0.06

[a] See Table 1 for strain identities.

[b] Induced or uninduced cells were obtained by growing them in acidogenic TSB+G (final pH 4.6 to 4.7) or nonacidogenic TSB−G (final pH 7.0 to 7.2) for 18 h at 37°C.

[c] Log (mean day-0 BHIA count) − log (mean day-7 BHIA count) for 0.0 kGy samples unirradiated controls).

[d] Calculated difference in log (mean BHIA counts for day-0 and day-7 samples based on the doses needed to reduce day-7 samples to the lower limit of detection (log CFU/ml = 1.0). This dose was calculated by analyzing the day-7 data using linear regression. This dose was then used to estimate the log count for day 0, again using linear regression. The value reported is the log (mean day-0 BHIA count) − log (mean day-7 BHIA count) for that dose.

<0.40 log cycle after 7 days of refrigerated storage (Table 3). Greater reductions (up to 1.68 log) during refrigerated storage were observed with many of the unirradiated TSB−G cultures, which would be indicative of the increased acid resistance of TSB+G-grown cells. Interestingly, the greatest decreases observed with the unirradiated TSB−G-grown cultures generally occurred at the higher

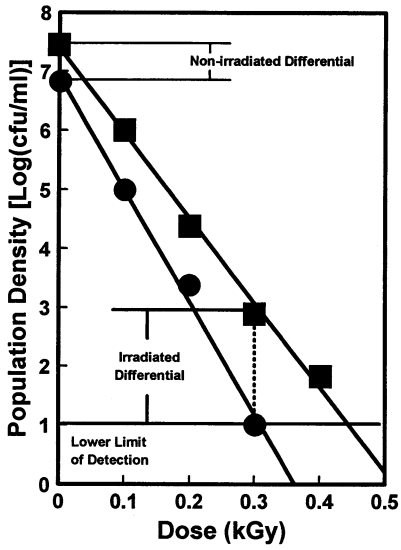

FIGURE 3. *The time-0 (●) and 7-day (■) survivor curve differentials for irradiated and unirradiated samples that were used to assess the ability of low-dose irradiation to enhance acid inactivation of enterohemorrhagic* Escherichia coli *during refrigerated storage.*

pH values. The extent of inactivation after 7 days of refrigerated storage of irradiated cultures was often greater than that of the corresponding unirradiated cultures (Table 3), particularly with the TSB+G-grown cells. Overall, the results suggest that irradiation may enhance to a limited degree the inactivation EHEC during subsequent refrigerated storage.

Injury. In addition to plating on nonselective BHIA, all samples were plated on MA agar. When exposed to a nonlethal stress, physiological injury to *E. coli* results in a loss of resistance to bile salts. Thus, the difference between the BHIA and MA counts can be used to measure of the number of injured cells. For example, a difference of log (BHIA count) − log(MA count) = 1.0 indicates that 90% of the viable cells are injured. This dual plating system was used to assess (i) the effect of BHI pH and prior induction of pH-dependent stationary-phase acid resistance on the extent of injury, (ii) the effect of refrigerated storage of the irradiated and unirradiated cultures at 2°C on the extent of injury, and (iii) the biological variability in these responses among the eight *E. coli* strains. Representative survivor curves based on BHIA and MA counts after 0 days and 7 days of storage at 2°C are presented in Figure 4.

The differences in log counts for BHIA and MA counts for the unirradiated (0.0 kGy) cultures were used to assess the extent of injury due to prior growth in the acidogenic and nonacidogenic media and subsequent exposure of the cells to moderately acidic BHI (Fig. 5). Differences in the pH of the BHI into which the *E. coli* isolates were transferred had little effect on the extent of injury in the time-0 samples. Immediately after inoculation, the extent of injury was small; log count differences were typically <0.3 for both the TSB+G (Fig. 5A) and TSB−G (Fig. 5B) cultures. Exceptions were S2 in TSB+G, S4 in TSB−G, and S1 in pH 5.0 and 5.5 TSB−G cultures, which had some-

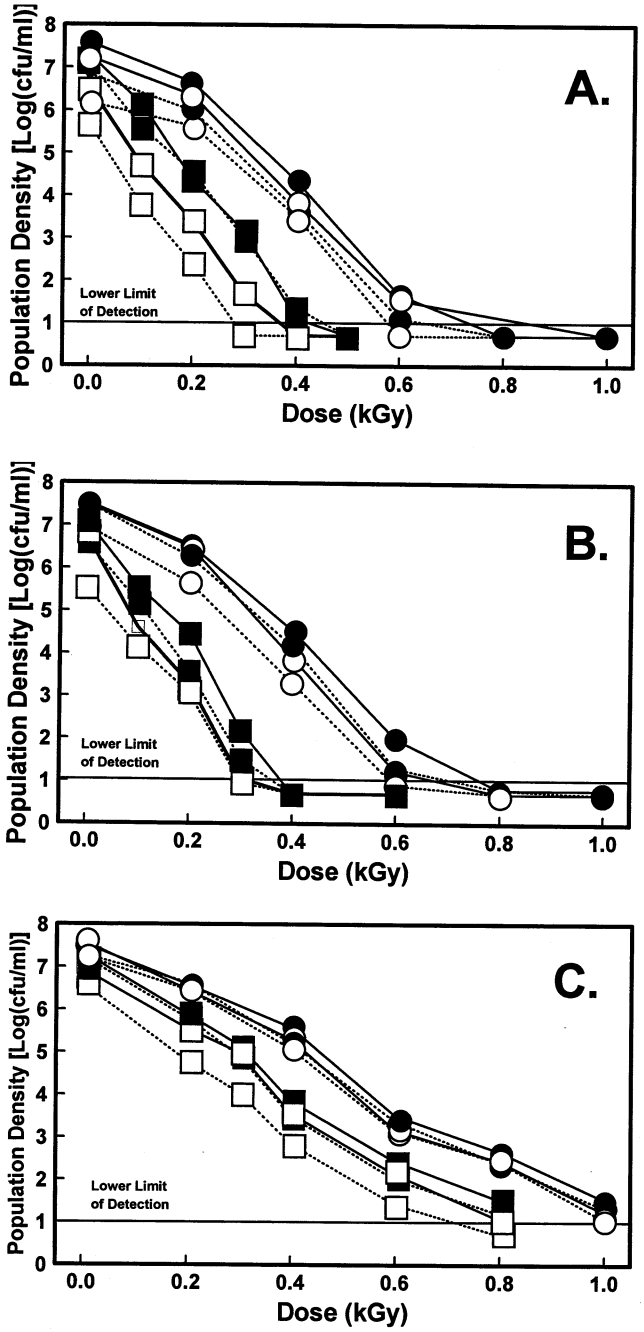

FIGURE 4. *Comparison of typical survivor curves based on brain heart infusion agar (BHIA) and MacConkey agar (MA) immediately after exposure of enterohemorrhagic* Escherichia coli *to low-dose irradiation and after 7 days of subsequent storage at 2°C: strain S2, pH 5.0 (A); strain S4, pH 4.0 (B); strain S5, pH 5.0 (C). TSB+G-grown (●, ○), TSB−G-grown (■, □); solid line = BHIA counts; dotted line = MA counts; open symbols = time 0; closed symbols = day 7. For the purposes of graphing, samples that fell below the lower limit of detection were assigned a value of 0.7.*

what elevated [log(BHIA) − log(MA)] differences. Counts of the stock cultures prior to inoculation (data not shown) indicated that the elevated level of injury observed with S2 and S4 occurred prior to transfer to BHI. The elevated level of injury observed with these strains when grown in an acidogenic medium has been observed previously *(5)*.

The extent of injury observed with the unirradiated TSB+G-grown (Fig. 5C) and TSB−G-grown (Fig. 5D) cultures after 7 days of refrigerated storage varied among strains and pH conditions. In general, the extent of injury was greater after 7 days of storage at 2°C, with this being more pronounced with the TSB−G-grown cells. For the TSB−G cultures, injury tended to be greatest at pH 4.0 and lowest at pH 4.5. Injury with the TSB+G cultures also was generally greatest at pH 4.0, while the pH that produced the least injury varied among the strains. No pattern was discernible among the TSB+G-grown EHEC cultures, but among the pH 4.0 TSB−G cultures the extent of injury corresponded to the relative acid resistance of the strains (e.g., the most acid sensitive strains, S4 and S6, had the greatest BHIA-MA differences).

An approach similar to that used to quantify the combined effect of pH and irradiation on the survival of EHEC was used to assess the relative importance of the variables on injury. The slopes of the survivor curves based on BHIA and MA counts were determined using linear regression. The dose that reduced the MA counts to the lower limit of detection (log CFU/ml = 1.0) was calculated and then substituted into the BHIA equation to estimate the corresponding viable count. The difference between the two counts was used as an estimate of combined effects of pH and irradiation (Fig. 6).

Quantitatively, the [log(BHIA) − log(MA)] differentials for the irradiated TSB+G and TSB−G cultures were generally less than 1.0 (i.e., less than 90% injury). Irradiation increased the extent of injury observed with the time-0 cultures of most strains (compare Fig. 5A and 5B and Fig. 6A and 6B), though there were several exceptions. One consistent exception was strain S7; irradiation appeared to decrease this strain's percentage of injured cells. Upon refrigerated storage, the percentage of injured cells observed with TSB+G-grown cells tended to decrease, particularly at pH 4.0 (compare Fig. 6A and 6C). With some exceptions, the extent of injury in the irradiated and unirradiated cultures after a week at 2°C was similar (compare Figs. 5C and 6C). This is in contrast to the results observed with the irradiated TSB−G-grown cultures; most strains showed increased injury after refrigerated storage (compare Fig. 6B and 6D). Injury tended to be greatest under the higher pH conditions. When compared against the corresponding unirradiated cultures, several strains had increased injury, and there was an apparent shift to maximal injury occurring at higher pH values (compare Figs. 5D and 6D).

DISCUSSION

The radiation resistances (*D* values = 0.05 to 0.18 kGy) (Table 2) for the eight strains examined in the current study support the conclusion that *E. coli* is relatively sensitive to ionizing radiation and can be controlled by low-dose treatments. Previously reported *D* values for *E. coli* under nonfrozen conditions have ranged from 0.06 to 0.41 kGy *(11, 12, 16, 41, 44)*. The current values fall at the lower end of this range, which is not surprising considering that the determinations were done in microbiological media. Radiation resistance is menstruum dependent, with resis-

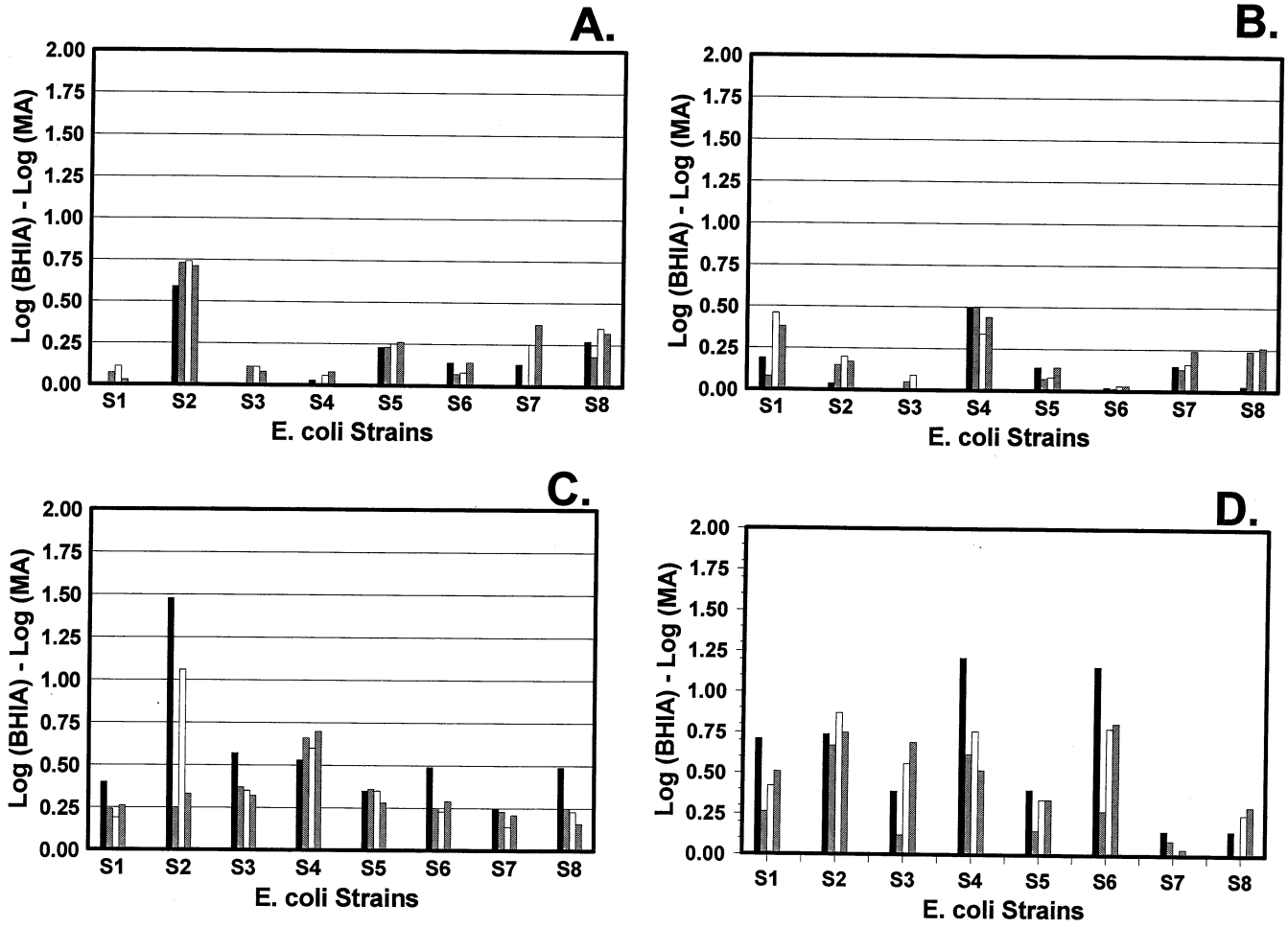

FIGURE 5. *Extent of injury observed with unirradiated cultures of enterohemorrhagic* Escherichia coli *based on differential in log counts on brain heart infusion agar and MacConkey agar: TSB+G-grown time 0 (A); TSB−G-grown time 0(B); TSB+G-grown, day 7 (C); TSB−G-grown, day 7 (D). Symbols: ■, pH 4.0; ▨, pH 4.5; □, pH 5.0; ▨, pH 5.5.*

tance increasing with the complexity of the food system. For example, the *D* value reported for an *E. coli* O157:H7 isolate irradiated in a 0.85% saline solution was 0.06 kGy *(12),* whereas the *D* values for *E. coli* irradiated in ground beef and chicken have been in the range of 0.24 to 0.28 kGy *(11, 41).*

There was substantial variability in the radiation resistances among *E. coli* strains, and this was evident with both the TSB+G-grown cultures (D = 0.63 to 0.175 kGy) and TSB−G-grown cultures (D = 0.45 to 0.124) (Table 2). The degree of variability was substantially greater than that observed by Thayer and Boyd (unpublished data) for a group of 16 pathogenic strains of *E. coli.* However, this is not surprising since the current strains were selected, in part, for the differences in their acid resistance *(5)* and were exposed to differing pH environments both before and during irradiation.

Prior studies have been inconclusive concerning the effect that pH has on the radiation resistance of *E. coli (2, 16).* The current results (Table 2) suggest that while pH differences between 4.0 and 5.5 can affect the radiation sensitivity of EHEC strains (Table 2), the overall impact of pH is relatively minor compared to the biological variability in the microorganisms' radiation resistance and the effects of prior growth conditions. Extrapolation of the results obtained with the current model system suggests further that the manipulation of pH would have limited impact in relation to the direct inactivation of EHEC in foods by irradiation. When the results of the 7-day samples are considered (Table 3), it appears that pH influences the ability of prior irradiation to increase the inactivation of EHEC during subsequent refrigerated storage. Reductions in EHEC counts as a result of 7-day storage at 2°C were consistently increased with irradiated pH 4.0 samples. Interestingly, increased inactivation during refrigerated storage as a result of irradiation also was observed at pH 5.5 for a number of strains. This effect was also noted with the unirradiated TSB−G-grown cultures of most strains. The reasons underlying this effect will require further research. Again extrapolating the results obtained with the current model system, irradiation in combination with pH reduction may be useful for enhancing the inactivation of EHEC during maturation of fermented products. For example, low-dose irradiation of fermented sausages immediately after completion of the initial fermentation period may enhance the inactivation of EHEC during the subsequent drying period.

The current results are based on the use of HCl as an acidulant. Hydrochloric acid is generally considered one of

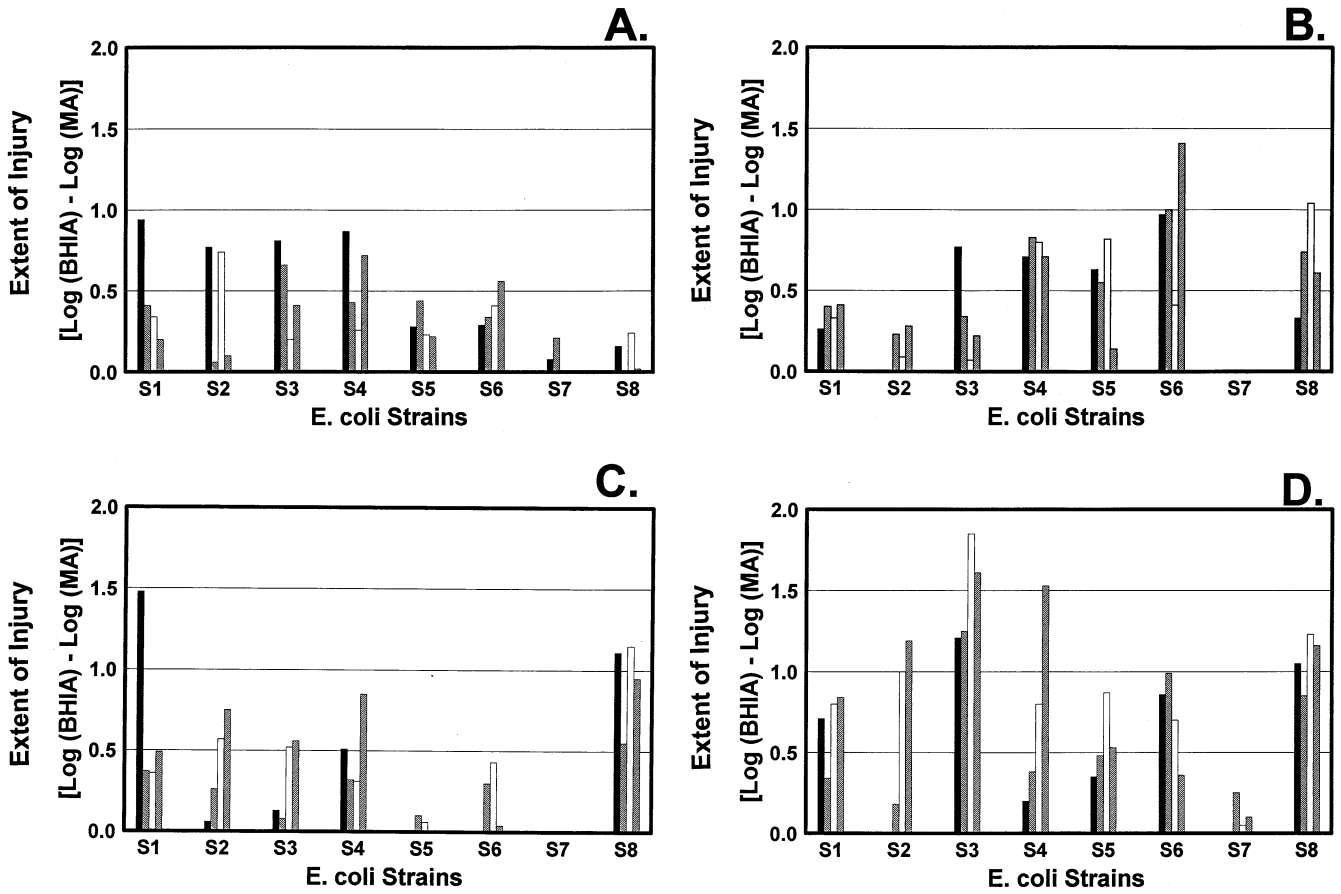

FIGURE 6. *Extent of injury observed with irradiated cultures of enterohemorrhagic* Escherichia coli *based on the differential in log counts on brain heart infusion agar and MacConkey agar (MA). The differential was estimated at the dose calculated to reduce the MA counts to the lower limit of detection based on linear regression of the survivor curves (see text). TSB+G-grown time 0 (A); TSB−G-grown time 0 (B); TSB+G-grown, day 7 (C); TSB−G-grown, day 7 (D). Symbols: ■, pH 4.0; ▧ pH 4.5; □, pH 5.0; ▨, pH 5.5.*

the gentlest acids in terms of its effect on microorganisms. It was used in the current study so that the effects of pH could be considered without the need to take into account the pH dependence of acid transport and subsequent anion effects associated with weak organic acids. It is likely that the organic acids used in foods (e.g., lactic acid, acetic acid) would have a greater impact on radiation resistance due to the anion effects associated with such compounds. Recently, Fielding et al. *(17)* reported that the radiation resistance of *E. coli* was decreased when irradiated in pH 4.6 microbiological medium containing acetic acid as compared with HCl-adjusted controls. Studies on the effects of acidulant identity on the radiation resistance of EHEC have been completed and will be reported separately.

Prior growth of EHEC in acidogenic TSB+G consistently increased the radiation resistance of all strains tested. This included both the isolates that had been identified previously as having inducible pH-dependent stationary-phase acid resistance and those that appeared to be constitutively acid resistant *(5)*. This suggests that either the pH-dependent induction of increased radiation resistance is mediated by a mechanism separate from that for acid resistance or that the duration of the acid challenge used by Buchanan and Edelson *(5)* was insufficient to detect the presence of pH-dependent stationary-phase acid resistance above the already extreme acid resistance afforded by the pH-independent *rpoS*-associated system in EHEC *(5)*. Studies just completed in our laboratory indicate that all of the strains used in the current study have both pH-independent and pH-dependent stationary-phase acid resistance (manuscript in preparation). It has long been recognized that stationary-phase cells are more resistant to ionizing radiation than exponential phase cells. Presumably, the increased radiation resistance observed with stationary-phase cells is associated with the expression of *rpoS*-regulated genes, and as such would be linked to the *rpoS*-associated, pH-independent stationary-phase acid resistance system in *E. coli*. This suggests further that the radiation cross-protection associated with growing the microorganism in acidogenic TSB+G is associated with the induction of the pH-dependent stationary-phase acid resistance.

The effects observed are consistent with other investigations where cross-protection against heat, UV radiation, and antimicrobials was afforded *E. coli* or *L. monocytogenes* after induction of acid resistance *(15, 22, 30)*. The mechanism by which pH-dependent stationary-phase acid resistance provides cross-protection will require additional investigations. Ionizing irradiation at temperatures above freezing has two effects: direct inactivation due to disruption of DNA and indirect effects due to the production of

hydroxyl radicals. Kim and Thayer *(24)* concluded that the primary effect of irradiation at 0°C was due to DNA damage and not cellular membrane disruption. However, the current study and previous investigations have found that ionizing radiation can injure various foodborne pathogens *(32, 42),* and effect that is generally correlated with damage to the cellular membrane. The induction of acid resistance appears to modulate the effect of ionizing radiation at either site. Acid habituation of *E. coli* decreases the extent of DNA damage by acid and enhances the cells' ability to repair DNA damage *(38).* Growth of *E. coli* in an acidic environment also increases the microorganism's resistance to UV light via a mechanism that appears not to involve the SOS DNA repair response *(22).* Both studies suggest that acid resistance could increase the radiation resistance of *E. coli* by enhanced repair of DNA. Alternatively, acid tolerance has been shown to provide *Salmonella* Typhimurium and *L. monocytogenes* cross-protection against a number of stresses that affect primarily the cellular membrane. Examples include increased resistance to heat, hydrogen peroxide, lactoperoxidase inactivation, crystal violet, osmotic stress, and ethanol *(15, 28, 30, 31).* Leyer and Johnson *(28)* concluded that the mechanism of acid resistance associated with cross-protection in *S.* Typhimurium involves changes in cell surface properties.

The current study is one of the few that has considered the effect of prior growth conditions on radiation resistance. Development of irradiation treatments that simultaneously optimize *E. coli* elimination and sensory attribute retention is dependent on having accurate measures of the microorganism's radiation resistance. Relatively small differences in reported versus actual irradiation *D* values have a significant impact on the efficacy of radiation treatments due to the exponential nature of radiation inactivation. The magnitude of the differences in radiation resistances observed in the current study could lead to substantial errors if a treatment based on the radiation resistance of EHEC that had not been induced for acid resistance was used to ensure elimination of isolates that had been induced for acid resistance. It is unlikely that a processor would be able to readily determine if a food contains induced or uninduced acid-resistant EHEC. Therefore, it would be prudent to base irradiation treatments on the microorganism in its most resistant state. Since inducible acid resistance has been observed with a number of foodborne pathogens, it seems worthwhile to recommend that currently published irradiation *D* values for various pathogens in different foods be reexamined to determine if cross-protection resulting from the induction of pH-dependent stationary-phase acid resistance further increases radiation resistance. Work is currently underway to determine the importance of cross-protection effects in moderately acidic foods.